SUPPLEMENTARY NOTE

Htt103Q Expression Levels

In order to analyze the level of Htt103Q protein expression in the suppressor strains, protein extracts were analyzed by immunoblotting. SC –Ura GAL yeast cultures were inoculated from overnight SC –Ura raffinose (RAF) cultures to an OD_{600} ~0.4, and were grown under inducing conditions for 5-6 hours. Cells were harvested by centrifugation and cell pellets were lysed by mechanical disruption with glass beads. Acid washed glass beads (425-600 μm, Sigma, St. Louis, MO) and lysis buffer (50 mM Tris pH 7.5; 100 mM NaCl; 1 mM EDTA; 50% Glycerol; 10 mM $MgCl_2$; 1X protease inhibitor cocktail) were added to the cell pellets, followed by vortexing three times for 5 min each, with 5 min incubations on ice in between. Glass beads and cell debris were removed by centrifugation and protein extracts were quantified using the BioRad Protein Assay (BioRad, Hercules, CA). Extracts were then mixed with 4x NuPage LDS Sample Buffer (Invitrogen, Carlsbad, CA), boiled 5 min, and subject to electrophoresis in 10% SDS-polyacrylamide gels. The proteins were transferred to nitrocellulose membranes and blotted as previously described [1]. Monoclonal GFP antibody (BD Biosciences, Palo Alto, CA) was used at a 1:1000 dilution, while polyclonal Rnq1 antibody was used at a 1:2000 dilution. For detection of GFP-tagged Htt103Q, α-Mouse Ig horseradish peroxidase conjugated antibody (Amersham Bioscience, Piscataway, NJ) was used at a dilution of 1:1000 followed by ECL (Amersham Bioscience, Piscataway, NJ), and exposure to X-ray film. Some variation of Htt103Q levels was detected by immunoblotting, likely due to the following: variable levels of aggregation of Htt103Q amongst the suppressor strains

likely affecting epitope recognition, variable copy number due to the pYES2 vector (a high-copy 2-micron plasmid) in which copy number varies from one transformant to another, and variation in the growth rates of the strains. Due to these factors, strains were determined to be false positives only if detectable levels of Htt103Q by immunoblotting were drastically reduced (Supplementary Figure 1a). In the case of Rnq1 protein, detection was done using α-rabbit Ig horseradish peroxidase conjugated antibody (Amersham Bioscience, Piscataway, NJ) at a dilution of 1:1000 followed by ECL and exposure to X-ray film.

Determination of RNQ prion status

The Rnq1 prion status has been shown to be critical for aggregation and toxicity of Htt103Q [2]. In order to confirm that the suppression of toxicity seen in the suppressor strains is not due to loss of the prion form of Rnq1, we analyzed pellet (prion form) and supernatant (soluble form) fractions from several of the suppressor strains expressing Htt103Q for the presence of Rnq1 (Supplementary Figure 1b). 25 ml cultures of suppressor strains were grown to approximately an OD_{600} 1.0 in SC –Ura GAL media, at which point cells were harvested by centrifugation at 2800 RCF for 5 min. Cell pellets were washed with 10 ml water chilled on ice, and spun as above. The cell pellets were resuspended in 200 μl lysis buffer (100 mM Tris pH 7.0, 200 mM NaCl, 1mM EDTA, 5% glycerol, 0.5 mM DTT, 1X protease inhibitor cocktail) and transferred to microfuge tubes. The cells were lysed by addition of acid washed glass beads (425-600 μm, Sigma, St. Louis, MO), vortexing for 1 min, addition of 200 μl of RIPA buffer (50 mM Tris pH 7.0, 200 mM NaCl, 1% Triton, 0.5 % Na-deoxycholate, 0.1% SDS), and further

vortexing for 10 sec. To eliminate the glass beads, the bottom of the microfuge tubes were punctured with a 20 gauge needle, and the contents were spun into a fresh tube at 3000 RCF for 15 sec. 60 μl of this supernatant was used as the "Total" sample, while 200 μl of the supernatant was centrifuged for 30 min at 80,000 rpm in the Beckman TLA 100 Ultracentrifuge. 60 μl of the supernatant from this step was isolated and used at the "Soluble" fraction, while the "Pellet" fraction was prepared by resuspending the pellet in 100 μl of lysis buffer and 100 μl of RIPA buffer, adding 67 μl of 4X protein sample buffer (SB), and boiling for 5 minutes. 20 μl of 4X SB was added to the "Total" and "Soluble" fractions, and boiled for 5 min. The samples were resolved by SDS-PAGE and immunoblotting as described above. All of the deletion strains analyzed retained Rnq1 in the prion state (pellet fraction) while expressing Htt103Q (Supplementary Figure 1b). We could therefore conclude that a change in the prion state of Rnq1 is not relieving Htt103Q-mediated toxicity in the isolated suppressors.

Literature Cited

1. Giorgini, F., Davies, H.G. & Braun, R.E. Translational repression by MSY4 inhibits spermatid differentiation in mice. *Development* **129**, 3669-79 (2002).
2. Meriin, A.B. et al. Huntington toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1. *J Cell Biol* **157**, 997-1004 (2002).